

PATENT ABSTRACTS OF JAPAN

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(54) PREPARATION OF LABORATORY ANIMAL

(57)Abstract:

PROBLEM TO BE SOLVED: To efficiently prepare a laboratory animal useful for the research of diseases such as obesity and hypertension, etc., by detecting a gene to be the cause of a specified phynotype.

SOLUTION: A restriction endonuclease cut part provided with a gene variation to be the cause of the phenotype selected from the obesity, the hypertension, diabetes, aging, hyperglycemia, arteriosclerosis, rheumatism and incomplete immunity is decided, DNA is extracted from an animal and processed by the restriction endonuclease, if the laboratory animal is homozygous, heterozygous or normal relating to the variation gene is decided based on the presence of a restriction fragment by the restriction endonuclease and the laboratory animal for expressing the phenotype is prepared.

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CLAIMS

[Claim(s)]

[Claim 1] An approach including detecting the gene variation which is the creation approach of the laboratory animal for the model system which discovers specific phenotype, and causes this phenotype.

[Claim 2] The method according to claim 1 of detecting restriction fragment length polymorphism for gene variation as an indicator.

[Claim 3] Furthermore, an approach according to claim 1 or 2 including sorting out a gay, a hetero, or a normal individual about this gene variation, and crossing this hetero individual and breeding it.

[Claim 4] At least the restriction-enzyme cutting section including the gene variation constituting the cause including the following processes of the creation approach:(1) this phenotype of the laboratory animal for the model system which discovers specific phenotype is determined, DNA is extracted from (2) animals, it processes with the restriction enzyme in (1), and this animal determines normal any [a gay, a hetero, or] they are about a mutant allele based on existence of the restriction fragment by (3) this restriction enzyme.

[Claim 5] Furthermore, an approach including making the individual which is a hetero cross, repeating process (1) - (3), and sorting out the individual of a hetero or a gay according to claim 4.

[Claim 6] The method according to claim 5 of performing a process (3) by the polymerase chain reaction-restriction-fragment-length-polymorphism method (PCR-RFLP law).

[Claim 7] The approach according to claim 1 to 6 by which specific phenotype is chosen from obesity, hypertension, diabetes mellitus, aging, hyperlipidemia, arteriosclerosis, rheumatism, and immune disorder.

[Claim 8] An approach including detecting the gene variation which is the approach of screening the model animal of obesity or II type <2> diabetes mellitus, and causes obesity or II type <2> diabetes mellitus.

[Claim 9] The approach according to claim 8 of performing by the PCR-RFLP method.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention relates to the creation approach of a laboratory animal used for the model system which discovers specific phenotype, and a model system mainly useful to research of a human disease etc. In more detail, out of many animals, it sorts out promptly and the target animal is related with accuracy and the method of obtaining a laboratory animal efficiently.

[0002]

[Description of the Prior Art] In order to perform effectively break through of various manifestation devices of a disease, and its therapy and development of the prevention approach, it is indispensable to use a suitable model system. Although the laboratory animal used for the model system relevant to hypertension, diabetes mellitus, obesity, aging, hyperlipidemia, arteriosclerosis, rheumatism, immune disorder, etc. is offered until now, the laboratory animal about the target phenotype is efficiently chosen from many candidates, and the approach of creating is not established. On the other hand, existence of gene variation leading to various phenotypes has been clarified with development of a gene-analysis technique. When the gene which does not have m (in the case of a recessive gene) and variation for the gene which has the variation constituting the cause of a certain phenotype (an example, disease) on a chromosome is expressed with +, the individual which has discovered the characteristic by this mutant allele is only a m/m individual which has variation in a gay. However, many of gay individuals may have very weak fecundity in being sterility, and although such a variation animal is useful as a model animal, it is unsuitable to propagation. Therefore, in order to obtain the gay individual which has specific phenotype, it is most efficient to cross the individuals (m/+) which have variation in a hetero, and to choose a m/m individual from the **. However, conventionally, since the

appearance up distinction of a hetero individual and the normal (+/+) individual without variation could not be carried out, it had to judge based on the phenotype of ** which had it produced normal any [a hetero or] the genotype of a certain individual was (progeny test). Therefore, the object of propagation was made to cross without distinguishing a hetero individual and a normal individual, the approach of choosing the individual which has discovered a gay's characteristic from produced ** was taken, and many parent animals were need. Moreover, even if genotype was a gay, it also had the model system which cannot detect the manifestation of a characteristic easily, and there was a problem also in the dependability of a genotype judging in such a case. Moreover, although the individual (+/+) which has the hetero individual (m/+) and the normal gene of a brood as control was used in the research using a m/m individual, since these hetero individual and a normal individual were undistinguishable like the above, it had to use as a +/- individual, genotype had to be distinguished by the progeny test, and it was uneconomical also in respect of time amount and an effort.

[0003] for example, the case of the obesity mouse used as a model system of the obesity in Homo sapiens -- the obesity -- the thing in at least six pairs of loci is known as a single gene variation which determines a characteristic, and all are mapped on autosome. The obesity with the obese (ob) gene whose number is one was already reported in 1950, and C57BL / 6 J-ob congenic mouse which mainly makes C57BL/6J a heredity background are used as a model animal of obesity and II type <2> diabetes mellitus after that. Cloning of the ob gene was done by Y.Zhang and others (Y. Zhang et al., Nature 372 1994, 425-432) in 1994, and the base sequence of the cDNA is determined. This ob gene is a single recessive gene located in the 6th chromosome, and it is known that the variation in this gene will bring the same phenotype as human obesity and II type <2> diabetes mellitus to a mouse. By the way, since it was difficult to judge a hetero individual and a normal individual from appearance conventionally, these were made to cross at random, although the ob/ob obesity individual which has this gene in a gay needs to choose what has ob gene from a normal individual on appearance for obtaining an obesity individual at a hetero (ob/+) and it needs to cross it, since a sex is sterility. Moreover, by research which uses such an ob/ob mouse like the above, the +/- mouse of a brood was used as control, and when it needed to be judged any of a hetero or a normal individual they are, it had to judge by the progeny test. Thus, conventionally, an effort and time amount great for manufacture of a laboratory animal and a check were required, and it was inefficient-like. In addition, also when not only when a mutant allele is recessive, but the mutant allele of these problems was dominant (M) (the individual which discovers the characteristic by this mutant allele is M/+ which has variation in M/M which it has in a gay, and a hetero), it

existed and waited for the solution.

[0004]

[Means for Solving the Problem] This invention solves the above-mentioned technical problem, it is the efficient creation approach of the laboratory animal for the model system which has specific phenotype, and an approach including detecting the gene variation leading to this phenotype is offered. this invention approach creates a desired laboratory animal by detecting the existence of gene variation which causes the target phenotype using typing of the gene by the molecule biological technique. For that purpose, the approach of arbitration used for detection of the gene which has variation in the field of molecular biology is employable. For example, the approach of using restriction fragment length polymorphism (restriction fragment length polymorphism, RFLP) as an indicator and the Southern hybridization method using a specific probe based on the existence like the restriction enzyme cutting section, the PCR-SSCP (Single Strand Conformation Polymorphism) method, etc. can be mentioned.

[0005] The phrase "laboratory animal" Becoming points out the animal for an experiment used for the model system about specific phenotype among this description. A "model animal" is an individual which has discovered specific phenotype among laboratory animals, and points out the individual which has a mutant allele leading to this phenotype in a "gay" or a "hetero" (in the case [Especially] of a dominant gene). Therefore, the individual which has a mutant allele in a gay, and not only the individual that it has in a hetero but all the normal individuals are contained in the above-mentioned laboratory animal.

[0006] Although typing of the gene by the molecule biological technique is used for this invention approach like the above, the approach using a RFLP indicator is desirable. Detection of RFLP can be performed by the approach known by the technical field concerned, and there are the Southern-blotting method, the approach of combining magnification of the specific field of DNA by polymerase chain reaction (polymerase chain reaction, PCR), restriction enzyme processing, and electrophoresis, etc. In the case of the latter, since there is no need of carrying out radiolabeling, it is desirable from viewpoints, such as safety. Although a certain individual can use various RFLP indicators for this invention approach a condition [a useful thing] judging whether it is the individual which has the mutant allele which caused specific phenotype in a gay or a hetero, it is desirable to choose a RFLP indicator with which the variation of a gene is included at least in the restriction enzyme cutting section. If such a RFLP indicator is specified, it can be easily determined normal any [a gay, a hetero, or] this animal is by processing DNA obtained from the experimental animal with a corresponding restriction enzyme, and checking existence of a restriction fragment by electrophoresis. The thing

(m/m, M/M, M/+) with the obtained gay or hetero individual is useful as a model animal, and its thing (m/+) and normal individual with a hetero individual are useful as a control animal of research. Furthermore, the descendant of a gay individual can be efficiently created by crossing a hetero individual.

[0007]

[Embodiment of the Invention] The creation of the laboratory animal for the model system which has specific phenotype by this invention approach usually includes the following processes.

(1) Determine at least the restriction enzyme cutting section including the gene variation leading to this phenotype, and extract DNA from (2) animals, process with the restriction enzyme in (1), and this animal determines normal any [a gay, a hetero, or] they are about a mutant alle based on existence of the restriction fragment by (3) this restriction enzyme. Furthermore, many target laboratory animals can be obtained by making the individual which is a hetero cross and repeating process (1) – (3). Existence of the restriction fragment in the above-mentioned process (3) can be performed based on the number and/or size of a restriction fragment. Preferably, this process (3) about detection of RFLP is performed combining magnification of the suitable field by PCR, restriction enzyme processing, and electrophoresis.

[0008] Although this invention approach has a useful variation of a gene to creation of the laboratory animal used for the model system relevant to the phenotype which is the cause, as such a model system, there are obesity, diabetes mellitus, hypertension, aging, hyperlipidemia, arteriosclerosis, rheumatism, immune disorder, etc. Moreover, although especially the class of animal is not restricted, a mouse, a rat, a rabbit, etc. can be illustrated. Although an example is given to below and this invention is explained to it in more detail, these do not limit this invention.

[0009]

[Example]

example 1 the same obesity developed in C57BL / 6 J-ob mouse, and Shionogi which are used as obesity of the creation Homo sapiens of the laboratory animal of the model system about obesity, and a model animal of II type <2> diabetes mellitus — the FLS-ob mouse which has a characteristic was used. the individual which each of these animals has variation in the obesity gene, and has a mutant alle to a gay — obesity — a characteristic is discovered. In addition, ob means the obesity gene with variation by the following explanation.

(1) According to the attached description, DNA was extracted from the tail or liver of an extract experimental animal of DNA using the DNA extract kit (QIAamp Tissue Kit) to this kit. In the case of the animal used for propagation, the head of a tail was cut 0.5cm and it used for the DNA

extract. Breeding of an examinee was continued for the wound after seal with adhesive, and the genotype [$+/+$ (normal), ob/ob (gay), and $ob/+$ (hetero)] of each experimental animal was judged by the progeny test except the obesity individual. The decision of a suitable variation array, magnification of a field including the variation by PCR, restriction enzyme processing, and detection of the restriction fragment by electrophoresis performed typing of ob gene by the molecule biological technique as following.

[0010] (2) obesity of a RFLP indicator given in decision reference (Y.Zhang et al., Nature 372 1994, 425-432) -- the variation array in the nucleotide number 427-431 was used as an indicator out of the gene variation which determines a characteristic. It is only the cutting section according [this variation array (CTGAG)] to a restriction enzyme DdeI. Therefore, although DNA obtained from the individual which has this mutant allele is cut by DdeI, since DNA which has a normal array (CCGAG) is not cut, it is predicted that it is useful as a RFLP indicator.

[0011] (3) Based on the base sequence (Y.Zhang et al., above-shown) of the ob gene cDNA by which the PCR method announcement was carried out, the primer 1 (=forward primer) and the primer 2 (=reverse primer) were set up and compounded to 5' side and 3' side of the RFLP indicator determined above (2). The nucleotide sequence of these primers 1 and 2 is shown in the array numbers 1 and 2 of an array table. Moreover, the relation between primers 1 and 2 and a RFLP indicator is shown in drawing 1. In drawing 1, the variation array is indicated by the array normal above an arrow head, and the lower part in relation to the array of the nucleotide number 427-431.

Therefore, in a mutant allele, there is at least the DdeI restriction enzyme cutting section of 427-431 at least in HaeIII each restriction enzyme cutting section of ScrFI of the nucleotide number 415-419, and 448-451 between a primer 1 and a primer 2. DNA 100-200 which extracted PCR from the experimental animal above (1) -- ng and Taq It carried out under existence of the above-mentioned primers 1 and 2 using thermal sequencer TSR-300 (Iwaki Glass) among reaction mixture of 20micro of whole quantity I containing the buffer (TAKARA SHUZO) containing DNA polymerase (TAKARA SHUZO) 0.5 unit, a dNTP mix (TAKARA SHUZO), and 1mM MgCl₂ (it is multistory about one drop of mineral oil). Conditions: Subsequently they are 73 degrees C and 5 minutes 30 cycles about 94 degrees C and 15 minutes [94 degrees C, 1 minute; 55 degrees C, 1 minute; 73 degrees C, and 1 minute]. Thus, the DNA field containing RFLP shown in drawing 1 of DNA extracted from each experimental animal was amplified using primers 1 and 2.

[0012] (4) The DNA fragment amplified by the restriction enzyme processing above (3) was processed according to the attached description with the restriction enzyme DdeI (Toyobo), and the existence of a RFLP indicator was

judged from the number of the bands which presented agarose gel electrophoresis, took a photograph, and appeared. First, after digesting 8micro of PCR products I in 37 degrees C by DdeI of 2-4 unit for 1.5 to 2 hours using the buffer for an attached reaction, agarose gel electrophoresis was presented with the digestive reaction mixture 4%, a photograph was taken also as that of UV irradiation after ethidium-bromide dyeing, and the band was observed. As contrast, using the unsettled PCR magnification fragment and the restriction enzyme ScrFI (Toyobo), in 37 degrees C, digestive treatment was carried out for 1.5 to 2 hours using the attached buffer for a reaction, and agarose gel electrophoresis was presented with the obtained reaction mixture like DdeI digestion. In addition, as negative contrast, sterile distilled water was used instead of DNA, and it processed similarly. A result is shown in drawing 2. Among drawing, an unsettled group, and 5, 6 and 7 express a DdeI processing group, and, as for 8, 9, and 10, as for a DNA size manufacturer, and 1, 2 and 3, M expresses a ScrFI processing group. As for +/+ (normal), and 2, 6 and 9, as for 1, 5, and 8, in each group, ob/ob (gay), and 3, 7 and 10 are DNA which that it is ob/+ (hetero) extracted from the checked individual in a progeny test. Moreover, 4 expresses negative contrast. The result was evaluated based on RFLP chosen as an indicator for detection of gene variation. That is, the genotype of each solid-state was judged in accordance with the following criteria with the number of bands.

+ /+ (normal); since a mutant allele does not exist, an PCR product is not cut by restriction enzyme processing, but the number of bands is one.

ob/ob (gay); since a mutant allele exists in both chromosomes, an PCR product is thoroughly cut by restriction enzyme processing, and the number of bands is two.

ob/+ (hetero); since a mutant allele exists only in chromosome of one of the two, the number of bands is three.

One band [in / clearly from drawing 2 / at 5 of a DdeI processing group / 140bp(s)] (normal); by two bands (gay); in 96bp(s) and 44bp(s), and 7, three bands (hetero) in 140bp(s), 96bp, and 44bp(s) are observed, and it is in agreement with the genotype determined by the progeny test at 6. On the other hand, when it processes with a restriction enzyme ScrFI, it turns out that change of a suitable band is not observed and it is not suitable for this invention approach.

[0013] Subsequently, using DNA extracted from about 600 C57BL(s)/6 J-ob, and FLS-ob mice, like the above, it inspected about existence of a RFLP indicator and compared with the result of a progeny test by the PCR method using primers 1 and 2, restriction enzyme DdeI processing, and electrophoresis. A result is shown in a table 1.

[A table 1]

obマウスの遺伝子型別成績

系 統		検 査 匹 数	ob/ob	ob/+	+/+
C57BL/6J-ob	♀	380	4	236	140
	♂	212	4	124	84
	小 計	592	8	360	224
FLS-ob	♀ *	28	14	14	0
	♂	20	0	14	6
	小 計	48	14	28	6
合 計		640匹	22匹	388匹	230匹

*コントロールとして使用

It could judge in all examples clearly from a table 1, and the result of a progeny test and the inequality were not accepted. Thus, the animal which the animal judged to be ob/ob (gay) was used as a model animal of obesity as it was, and was judged to be ob/+ (hetero) can be crossed further, and propagation can be presented with it. Moreover, the hetero and normal mouse of an obesity mouse and a brood are useful as control of the experiment which uses this obesity mouse.

[0014] Example 2 Like the creation example 1 of an obesity mouse, by the PCR method using primers 1 and 2, restriction enzyme DdeI processing, and electrophoresis, it inspected about existence of a RFLP indicator, and the individual judged to be a hetero is crossed, and was bred, and C57BL / 6 J-ob/ob mouse was created. The number of parent mice required in order to obtain 120 ob/ob mice per month is shown in a table 2 as compared with the case of the approach of making the conventional random crossing a hetero individual and a normal individual.

[A table 2]

遺伝子型別導入の繁殖面への寄与

C57BL/6J-ob/obマウス		120匹	／ 月供給の場合
従来法（後代検定法）			
♀ ob/+, +/+	510匹	}	532匹（100%）
♂ ob/+	22匹		
改良法			
♀ ob/+	320匹	}	334匹（63%）
♂ ob/+	14匹		
改良法、♀ 1:♂ 1同居			
♀ ob/+	200匹	}	400匹（75%）
♂ ob/+	200匹		

The improving method means among a table how (**1 animal and **2-3 animal are made to usually live together) to carry out living-together mating of **1 animal by which the approach and improving method **1:**1 living together which make only the individual judged to be a hetero cross were judged to be a hetero, and the **1 animal. although many parent mice are required clearly from a table 2 in the case of a conventional method since a normal mouse (+/+) is intermingled — PCR-RFLP of this invention — if law is used, since only the mouse of a hetero mold can be certainly used as a parent mouse, an ob/ob mouse is intentionally obtained from a small number of parent mice efficiently. Moreover, the time difference in the case where the genotype of ** obtained from the parents of a brood is judged by this invention approach, and the case of judging by the progeny test method is shown in a table 3.

[A table 3]

obマウスの遺伝子型別の時期

従来法（後代検定法）

ob/ob 5-6w 判定

ob/+, +/+ 10w → 13w → 18w → 21w → 26w
 (♀の場合) 交配 1回目の出産 仔の判定 2回目の出産 仔の判定

改良法

ob/ob, ob/+, +/+ 5-6w 判定（出生時以降、いつでも可能）

"A judgment of **" in a progeny test is performed among a table based on

the existence of ** of ob/ob. According to this invention approach, there is almost no time constraint in the judgment of an individual, and a laboratory animal can be created very efficiently so that clearly from a table 3.

[0015]

[Layout Table]

[0016] array number 1: -- die-length [of an array]: -- mold [of 20 arrays]:
-- number [of nucleic-acid chains]: -- single strand topology: -- class [of
straight chain-like array]: -- synthetic DNA array TCCAAGATGG
ACCAGACTCT 20 [0017] array number 2: -- die-length [of an array]: --
mold [of 20 arrays]: -- number [of nucleic-acid chains]: -- single strand
topology: -- class [of straight chain-like array]: -- synthetic DNA array
AGGGAGCAGC TCTTGGAGAA 20

[Translation done.]

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]

[Drawing 1] The mimetic diagram to which at least the restriction enzyme DdeI cutting section including the variation on ob gene of an obesity mouse indicates the relation of primers 1 and 2 to be a (RFLP indicator).

[Drawing 2] Copy drawing of the photograph which processed the DNA fragment amplified by PCR using the primers 1 and 2 shown in drawing 1 with restriction enzymes DdeI and ScrFI, presented agarose gel electrophoresis with the reaction mixture, and took a photograph of and obtained the migration pattern.

[Translation done.]